

H36.D2.B7 ANTI-TISSUE FACTOR LIGHT CHAIN VARIABLE REGION

GACATTCAGATGACCCAGTCTCCTGCCTCCAGTCTGCATCTCTGGGAGAAAGTGTCAACCATCATGCG
D I Q M T Q S P A S Q S A S L G E S V T I T C

CTGGCAAGTCAGACCATTTGATACATGGTTAGCATGGTATCAGCAGAAACCAGGAAATCTCCTCAGCTC
L A S Q T I D T W L A W Y Q Q K P G K S P Q L

CTGATTATGCTGCCACCAACTTGGCAGATGGGGTCCCATCAAGGTTCAAGTGGCAGTGGATCTGGCACA
L I Y A A T N L A D G V P S R F S G S G S G T

AAATTTTCTTTCAAGATCAGCAGCCTACAGGCTGAAGATTTTGTAATATT TACTGTCAACAAGTTTAC
K F S F K I S S L Q A E D F V N Y Y C Q Q V Y

AGTTCCTCATTACGTTTCGGTGTGGGACCAAGCTGGAGCTGAAA
S S P F T F G A G T K L E L K

FIG. 1A

H36.D2.B7 ANTI-TISSUE FACTOR HEAVY CHAIN VARIABLE REGION

GAGATCCAGCTGCAGCAGTCTGGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGCAGGTATCCTGCAAG
E I Q L Q Q S G P E L V K P G A S V Q V S C K

ACTTCTGGTTACTCATTCACTGACTACAAACGIGTACTGGGTGAGGCAGAGCCATGGAAAGAGCCCTTGAG
T S G Y S F T D Y N V Y W V R Q S H G K S L E

TGGATTGGATATATTGATCCCTTACAAATGGTATTACTATCTACGACCAGAACTTCAAGGGCAAGGCCACA
W I G Y I D P Y N G I T I Y D Q N F K G K A T

TTGACTGTTGACAAAGTCTTCCACCACAGCCCTTCATGTCATCTCAACAGCCCTGACATCTGACGACTCTGCA
L T V D K S S T T A F M H L N S L T S D S A

GTTTATTTCTGTGCAAGAGATGTGACTACGGCCCTTGACTTCTGTTGGGGCCAAAGGCACCACTCTCAGTC
V Y F C A R D V T T A L D F W G Q G T T L T V

TCCTCA
S S

FIG. 1B

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ANTIBODY	APPARENT K_1 , M^{-1}	APPARENT K_1 , M
BY ELISA		
D2	5.2×10^9	1.9×10^{-10}
I47	6.5×10^9	1.5×10^{-10}
K73	9.8×10^9	1.0×10^{-10}
K80	2.3×10^9	4.3×10^{-10}
L102	2.5×10^9	4.0×10^{-10}
L133	1.7×10^9	5.9×10^{-10}
BY BIACore		
H36	3.1×10^{10}	3.2×10^{-11}
I43	2.3×10^9	4.3×10^{-10}
I47	3.2×10^9	3.1×10^{-10}
L133	4.6×10^9	2.2×10^{-10}
M107	1.1×10^9	9.1×10^{-10}

FIG. 2

ANTIBODY NAME	% INHIBITION ANTIBODY PREINCUBATED WITH TF/VIIa
D1	0
D1B	1
H31	4
H36	95
I43	1
J131	7
K80	0
K82	0
K87	1
L97B	7
L101	0
L102	0
L105	0
L133	0
M5	1
M107	34

FIG. 3

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ANTIBODY NAME	<u>% INHIBITION</u> TF PREINCUBATED WITH ANTIBODY PRIOR TO ADDITION OF VIIa	<u>% INHIBITION</u> TF PREINCUBATED WITH VIIa PRIOR TO ADDITION OF ANTIBODY
D1	15	nd
D1B	48	12.7
H31	64	21
H36	0	0
I43	68	55
J131	38	11
K80	12	nd
K82	0	nd
K87	0	nd
L96	0	nd
L101	38	11
L102	14	nd
L105	4	nd
L133	13	nd
M5	0	nd
M107	0	nd

FIG. 4

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[rhTF], nM	[H36.D2], nM	H36.D2/rhTF MOLAR RATIO	CLOTTING TIME (SECONDS)	% INHIBITION OF rhTF FUNCTION
0.0048	0	0	102.3	0
	1.61	335.4	114.3	31.3
	3.23	670.8	121.3	45.8
0.023	0	0	77.6	0
	1.61	70.0	85.3	52.2
	3.23	140.0	91.1	65.2
0.092	0	0	77.6	0
	1.61	70.0	85.3	52.2
	3.23	140.0	91.1	65.2
0.46	0	0	77.6	0
	1.61	70.0	85.3	52.2
	3.23	140.0	91.1	65.2
2.30	0	0	77.6	0
	1.61	70.0	85.3	52.2
	3.23	140.0	91.1	65.2
11.52	0	0	77.6	0
	1.61	70.0	85.3	52.2
	3.23	140.0	91.1	65.2

FIG. 5

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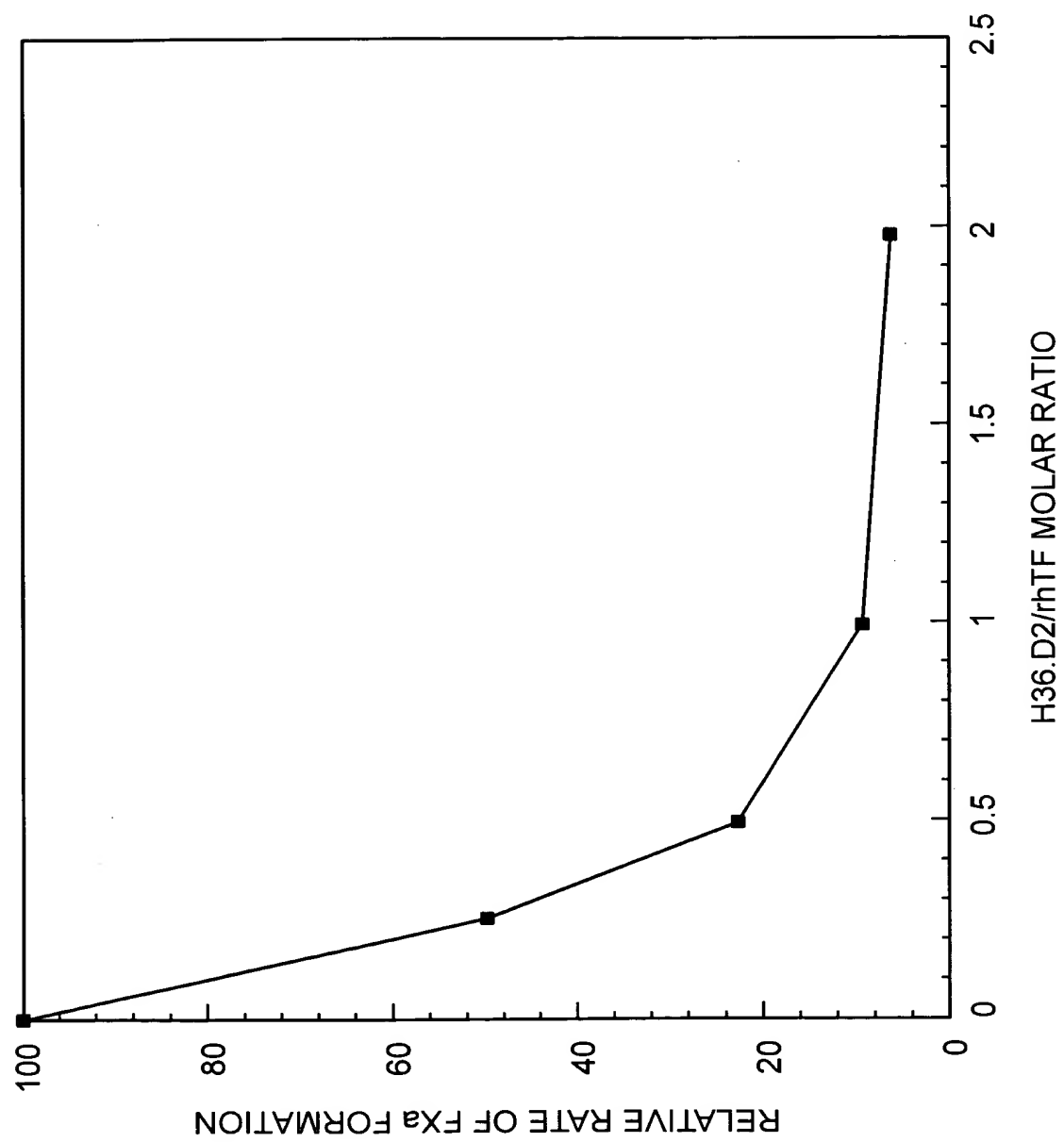


FIG. 6A

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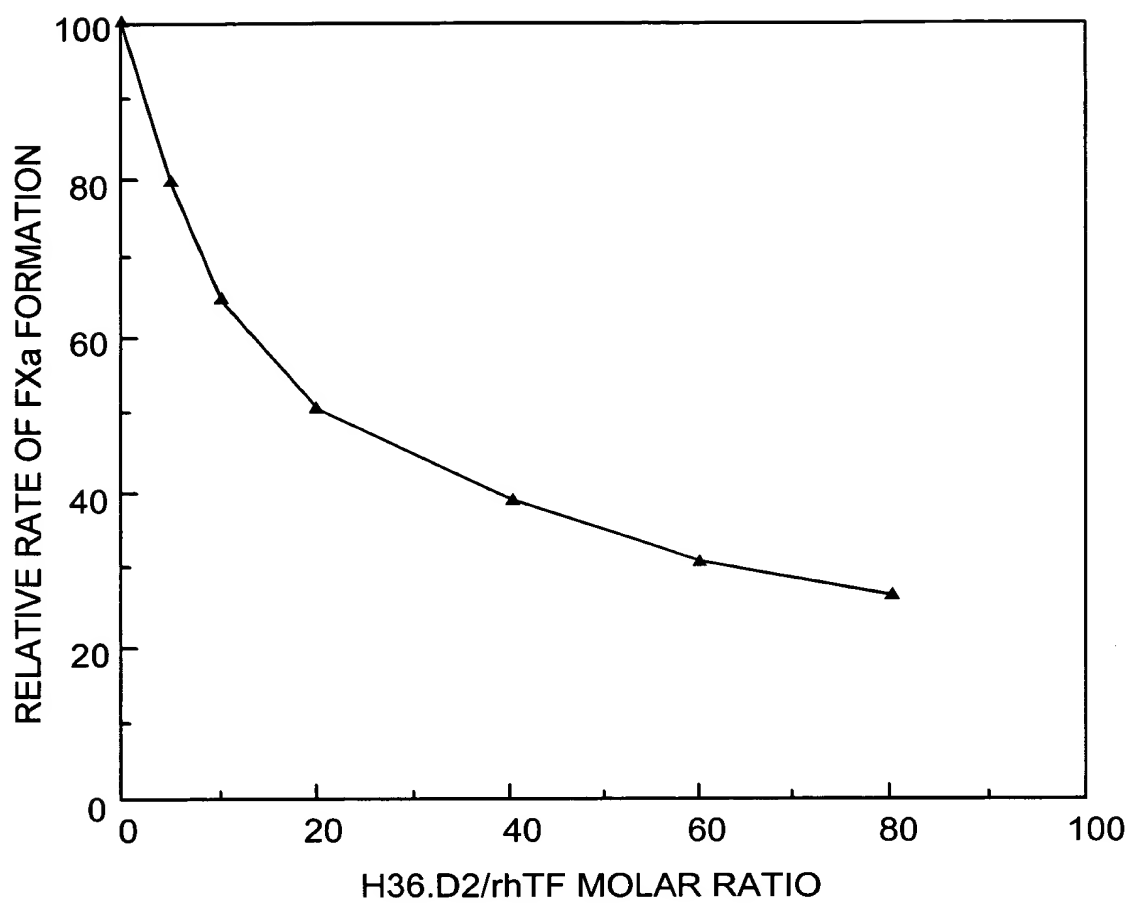


FIG. 6B

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H36.D2 CONCENTRATION (ng)	<u>% INHIBITION</u> CELLS (TF/FVII) AND H36.D2 PREINCUBATED PRIOR TO FX ADDITION	<u>% INHIBITION</u> FX AND H36.D2 ARE ADDED SIMULTANEOUSLY TO CELLS (TF/FVII)
0	0	0
50	88	nd
100	92	nd
200	97	nd
800	nd	76
1600	nd	78
3200	nd	92

FIG. 7

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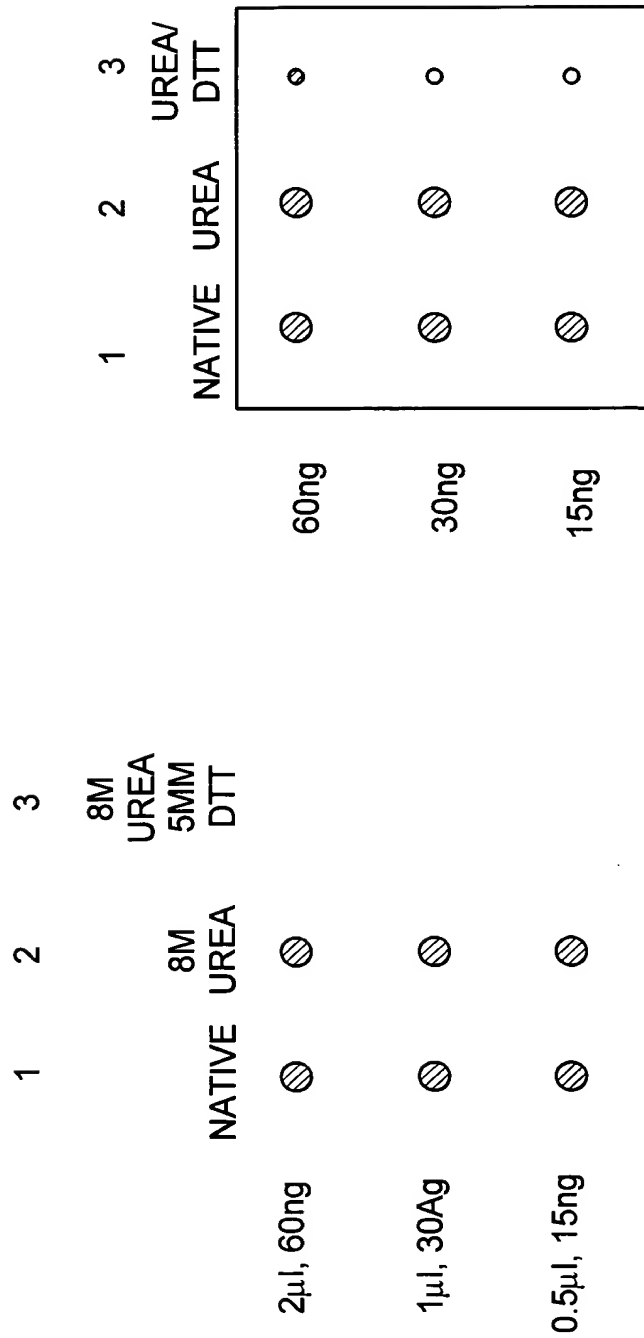


FIG. 8B

FIG. 8A

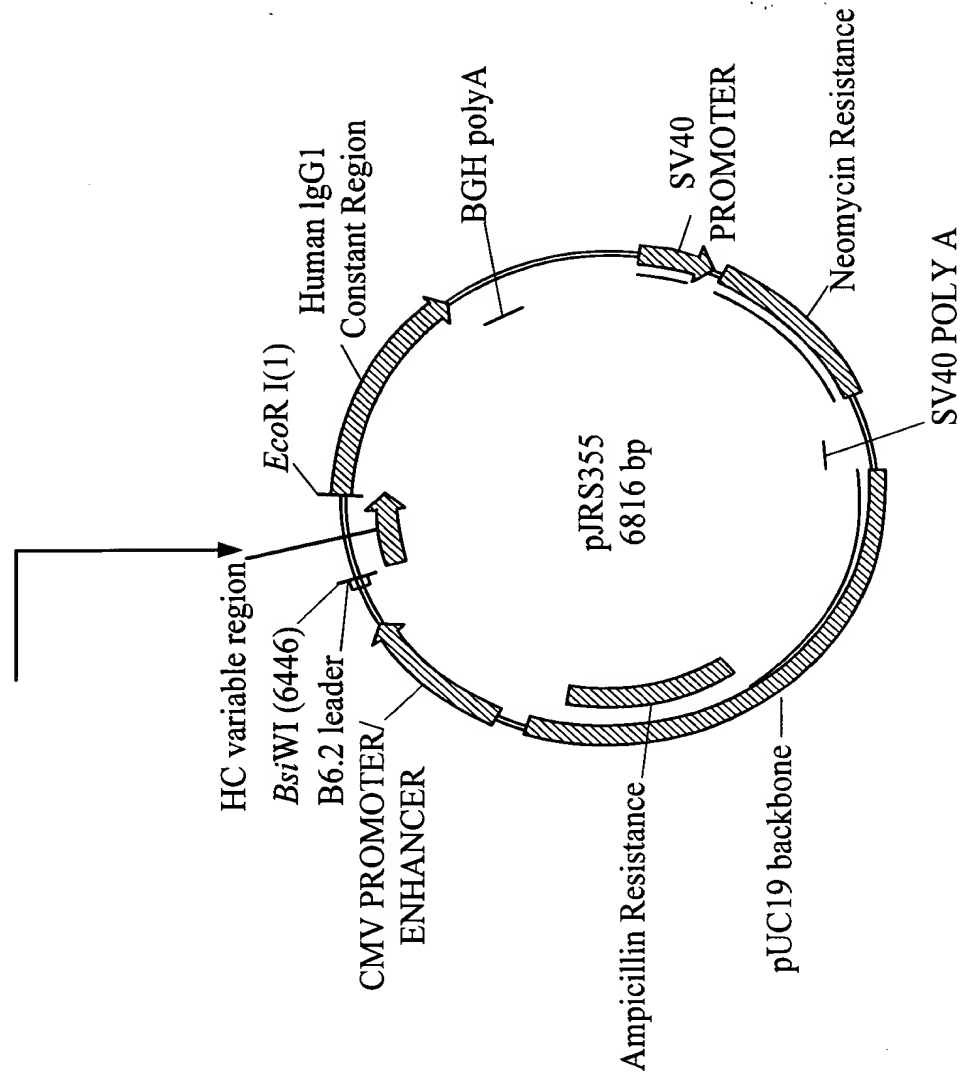


FIG. 9B

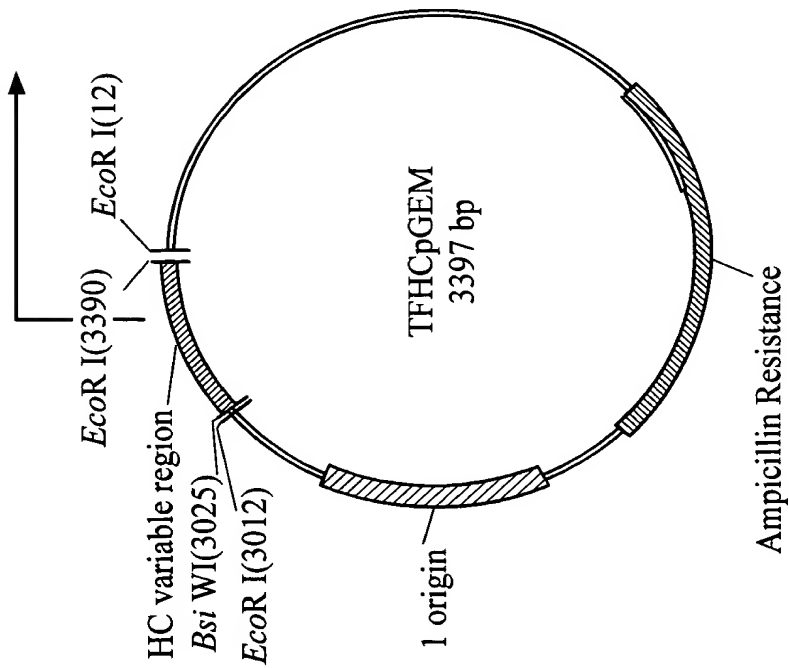


FIG. 9A

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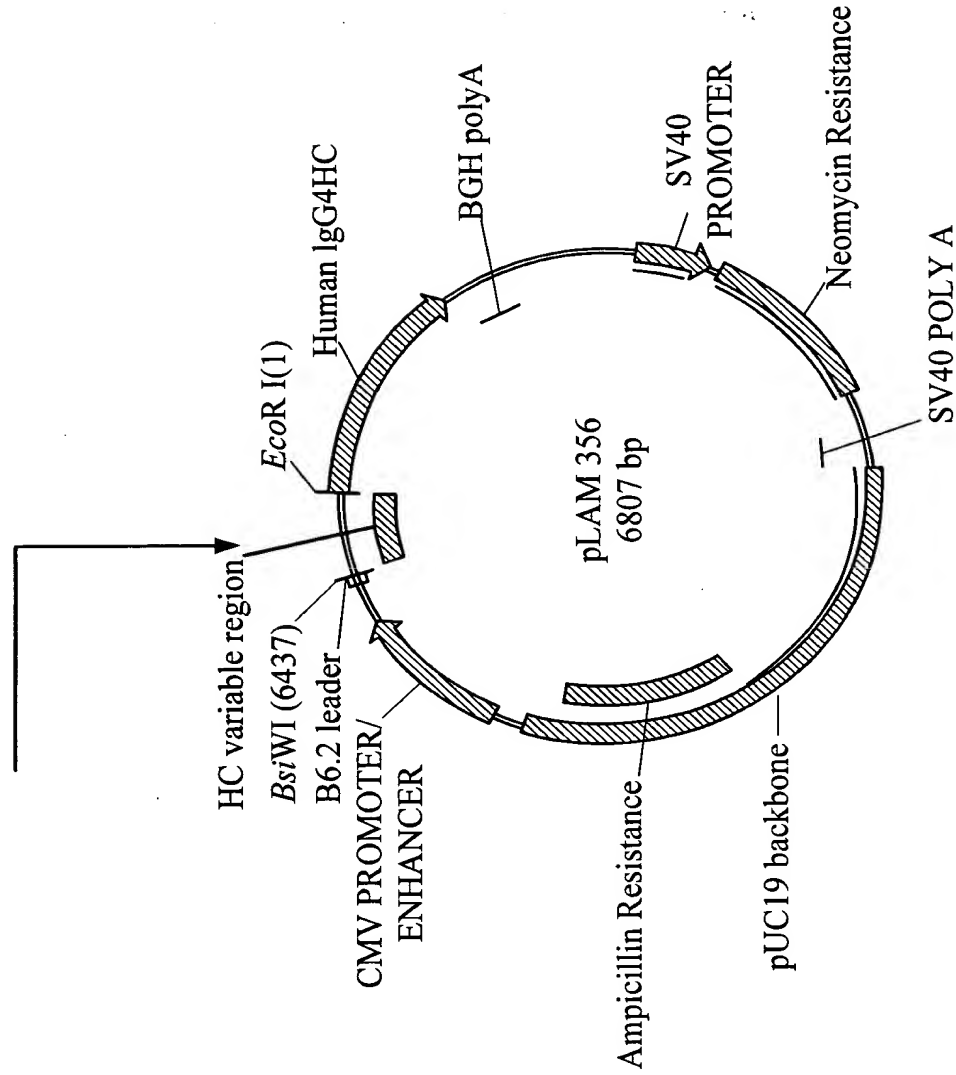


FIG. 9D

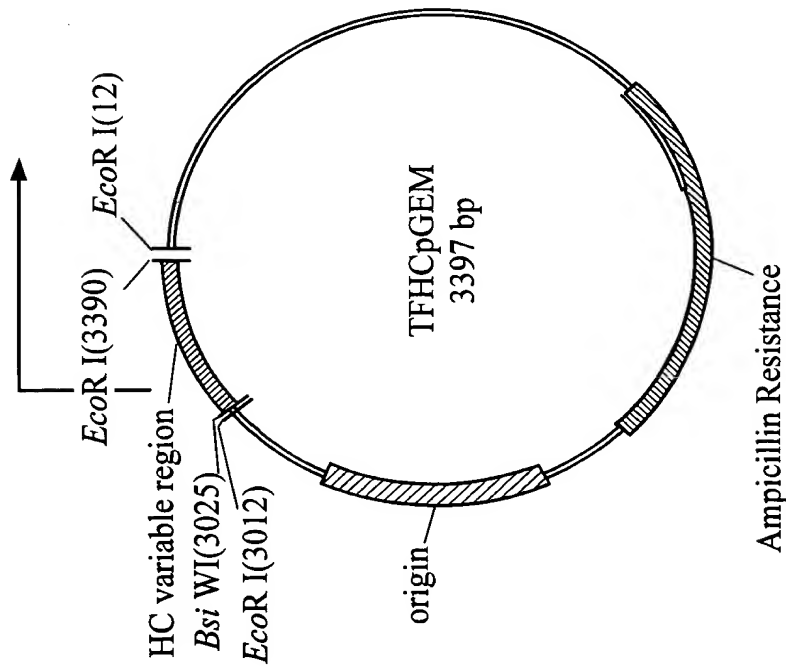


FIG. 9C

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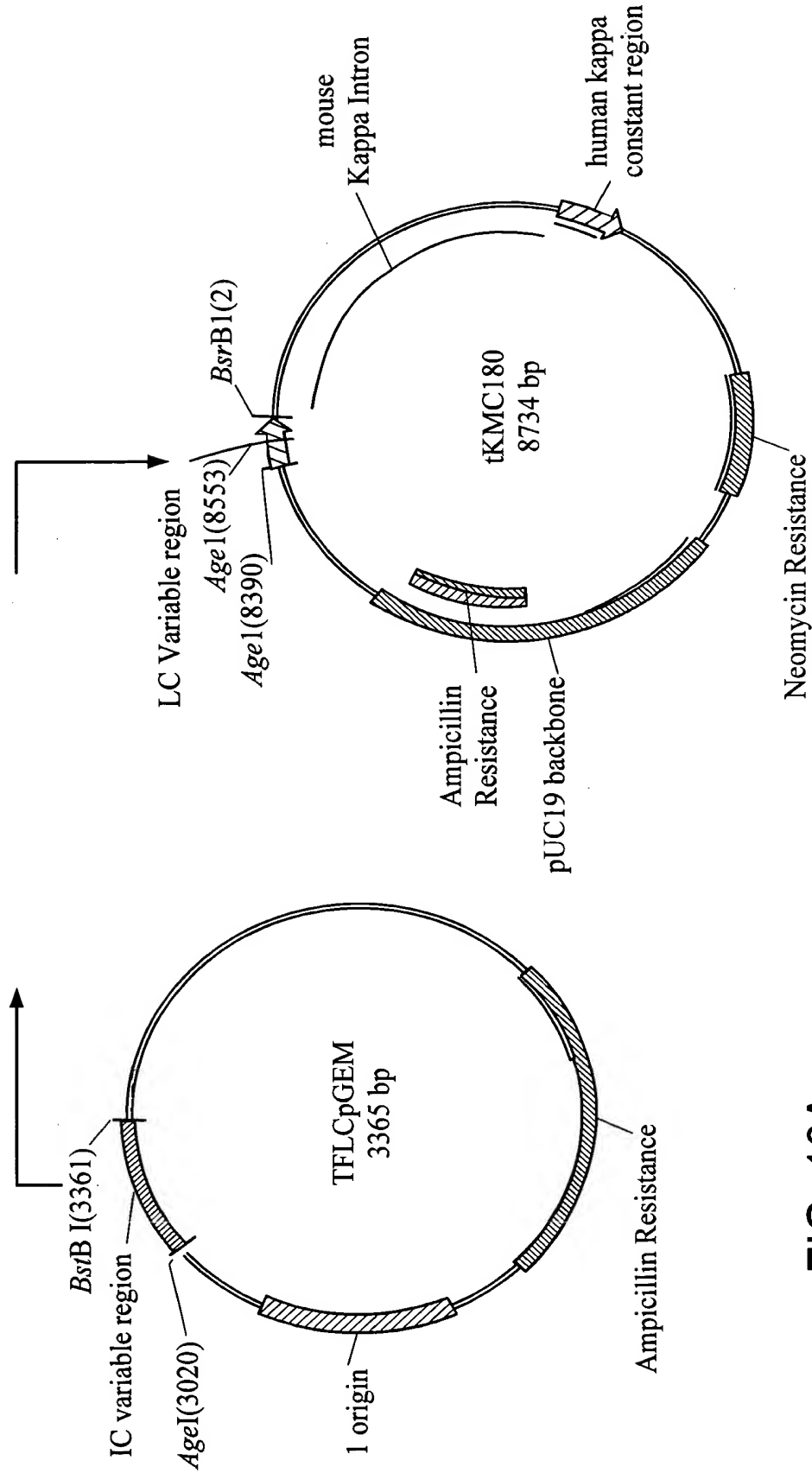


FIG. 10B

FIG. 10A

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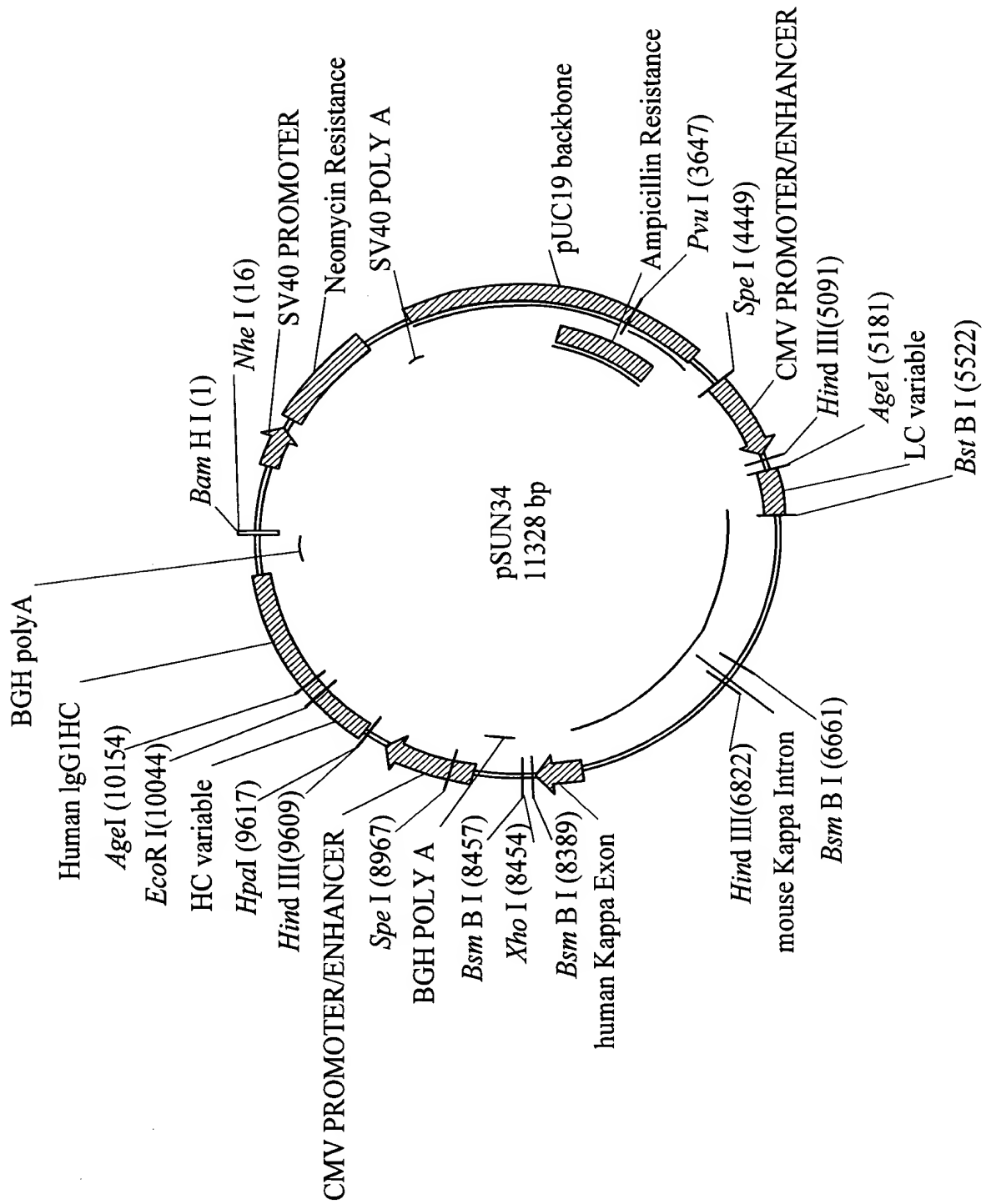


FIG. 11

Humanization of anti-Tissue Factor Antibody cH36

Sequences of Partially and Fully Humanized Light Chain (LC) Variable Regions

Light Chain (LC) FR Sequences

FR1 (23 AA)	FR2 (14 AA)	FR3 (32 AA)	FR4 (10 AA)	Names
1 10 20 35 47 57 60 70 80 86 98 107				
DIQMTQSPASQASLGE SVTITC WYQQKPGKSPQ LIY GVPSRFSGSGSGTKFSKISSLQAE DFVNY YC FGAGTKLE IK				cH36-LC
DIQMTQSPASQASLGE SVTITC WYQQKPGKSPQ LIY GVPSRFSGSGSGTKFSKISSLQAE DFVNY YC FGAGTKLE IK				LC-03
DIQMTQSPASQASLGE SVTITC WYQQKPGKSPQ LIY GVPSRFSGSGSGTKFSKISSLQAE DFVNY YC FGAGTKLE IK				LC-04
DIQMTQSPASQASLGE SVTITC WYQQKPGKSPQ LIY GVPSRFSGSGSGTKFSKISSLQAE DFVNY YC FGAGTKLE IK				LC-05
DIQMTQSPASQASLGE SVTITC WYQQKPGKSPQ LIY GVPSRFSGSGSGTKFSKISSLQAE DFVNY YC FGAGTKLE IK				LC-06
DIQMTQSPASQASLGE SVTITC WYQQKPGKSPQ LIY GVPSRFSGSGSGTKFSKISSLQAE DFVNY YC FGAGTKLE IK				LC-07
DIQMTQSPASQASLGE SVTITC WYQQKPGKSPQ LIY GVPSRFSGSGSGTKFSKISSLQAE DFVNY YC FGAGTKLE IK				LC-08
DIQMTQSPASQASLGE SVTITC WYQQKPGKSPQ LIY GVPSRFSGSGSGTKFSKISSLQAE DFVNY YC FGAGTKLE IK				LC-09
DIQMTQSPASQASLGE SVTITC WYQQKPGKSPQ LIY GVPSRFSGSGSGTKFSKISSLQAE DFVNY YC FGAGTKLE IK				LC-10
DIQMTQSPASQASLGE SVTITC WYQQKPGKSPQ LIY GVPSRFSGSGSGTKFSKISSLQAE DFVNY YC FGAGTKLE IK				LC-11
DIQMTQSPASQASLGE SVTITC WYQQKPGKSPQ LIY GVPSRFSGSGSGTKFSKISSLQAE DFVNY YC FGAGTKLE IK				LC-12

FIG. 12A

Light Chain CDR Sequences of cH36

CDR1 (11 AA)	CDR2 (7 AA)	CDR3 (9 AA)
24 34 50 56 97		
L A S Q T I D T W L A A A T N L A D Q Q V Y S S P F T		

FIG. 12B

FIG. 12C

FIG. 12D

hOAT (IgG1) CONSTANT REGIONS SEQUENCES

SEQUENCES OF LC CONSTANT:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLTLSKADYEKH

KVYACEVTHQGLSSPVTKSFNRGEC

FIG. 14A

SEQUENCES OF HC CONSTANT:

EEASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYIC

NVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSSHEDPEVKFNWYVDGVEV

HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYITLPPSRDELTKNQVSLTCL

VKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK

FIG. 14B

hFAT (IgG1) CONSTANT REGION SEQUENCES

SEQUENCES OF LC CONSTANT:

RTVAAPSVFI FPPSDEQLKSGTASVVCLLNFFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSLTLSKADYEK

HKVYACEVTHQGLSSPVTKSFNRGEC

FIG. 15A

SEQUENCES OF HC CONSTANT:

EFASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTTY

TCNVDPKPSNTKVDKRVESKYGPPCPCPAPEFLGSPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGV

EVHNAKTPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI SKAKGQPREPQVYITLPPSQEEMTKNQVSL

TCLVKGFYPSDDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSRLTVTDKSRWQEGNVFSCSMHEALHNHYTQKSLSLSLGK

FIG. 15B